

The University of Chicago

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A Submicroscopic Component of the Guinea Pig Liver Cell;
Its Significance in Glycogen Storage and the Regulation
of Blood Sugar

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

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COMPONENT OF THE GUINEA PIG LIVER CELL;
ITS SIGNIFICANCE IN GLYCOGEN STORAGE
AND THE REGULATION OF
BLOOD SUGAR ¹

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INTRODUCTION

The advent of the ultracentrifuge has given great impetus to the study of ultramicroscopic structures found in animal cells. Doctor Claude ('40) at the Rockefeller Institute and Professor Bensley and myself at the Hull Laboratories recently have been studying these ultramicroscopic structures.

We have found ² that the cytoplasm of the guinea pig liver contains two distinct submicroscopic particulates in addition to the mitochondria previously separated by Bensley and Hoerr ('34). One of these, particulate glycogen, is the subject of this paper. The second is a lipo-protein complex. This ultramicroscopic lipo-protein complex differs in chemical composition from the microscopically visible mitochondria previously analyzed by Bensley ('37). (The latter also contains proteins and fats.) Furthermore, the lipo-protein ultramicroscopic particles as well as the visible mitochondria contain some of the respiratory enzymes; Lazarow and Barrón ('41, also unpublished data). The enzyme phosphatase has been found by Kabat ('41) to be associated with the lipo-protein ultramicroscopic particle. Claude found that the chick virus

¹ This work has been aided by a grant from the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

² Data soon to be published.

tumor principle was associated with this normal ultramicroscopic components of cells. These facts suggest that the particulate component of protoplasm play an extremely important role in the physiological activity and organization of the cell.

HISTORICAL CONSIDERATION OF THE DISTRIBUTION OF GLYCOGEN IN THE CELL

Subsequent to his discovery of glycogen in 1857, Claude Bernard (1859) studied, by means of the iodine reaction, the distribution of glycogen in various tissues including liver, placenta, and various embryonic tissues. He described, for example, plaques of cells in the amniotic membrane of the calf containing amorphous and round granules of a brown staining material and pointed out their similarity to those of liver cells.

At the same time that Claude Bernard announced his separation of glycogen, Schiff (1857) erroneously announced that he could identify glycogen in the unstained liver as granules. These granules, which were clearly differentiated from fat droplets, were said to be abundant in glycogen-rich livers and scarce in livers containing little glycogen. He further claimed that they completely disappeared by digestion with amylase. However, Bock and Hoffmann (1872), showed that the granules described by Schiff were present in livers containing no glycogen whatever and that after staining with a solution of iodine in potassium iodide a diffuse brown staining network could be seen between the granules of Schiff, which themselves remained colorless. Furthermore, the iodine reaction disappeared after salivary digestion. Even before Bock and Hoffmann, Rouget (1859) said that in cartilage and muscle, the glycogen existed as a "plasma" and not as a granular substance.

Heidenhain (1883), referring to the work done in his laboratory in 1879 by Richard Kayser, states that in alcohol fixed sections, the glycogen exists in the form of granules and flakes regularly located upon one side of the cell. However, he does

not state whether he considers this distribution as an artifact of the alcohol fixation. Külz (1881) as well as many authors following him, Afanassjew (1883), Barfurth (1885), Best ('03); and Fischera ('04), pointed out that this granularity, localized at one side of the cell, was an artifact of the alcohol fixation.

To Ehrlich (1883), however, belongs the credit of conclusively proving that glycogen is uniformly distributed throughout the liver cell in a diffuse state. He reasoned that, if the glycogen granules were preformed, they should be demonstrable in the dried preparation. However, upon staining a dried smear of liver with iodine he observed a homogeneous brown staining of the cytoplasm; whereas the nucleus contained no brown color. This diffuse distribution of the glycogen was confirmed for cells in tissue culture of *Fundulus* embryo by Lewis ('21), and for liver by Nordman ('29). Gersh ('38), by means of the freezing-drying technique, obtained the same results that Ehrlich did.

The separation of glycogen in the form of submicroscopic particulates, in no way contradicts any of the above observations. A uniform distribution of the submicroscopic component throughout the cytoplasm of the liver cell would give a diffuse appearance when studied by the freezing-drying method. However, if glycogen possesses the ability to form submicroscopic³ aggregates, it may also be able to form microscopically visible structures. Marchand (1885), conceded the diffuse distribution of glycogen in liver, but observed vacuoles of glycogen in dried preparations of leucocytes. Similarly, Gierke ('05), observed glistening balls in fresh cartilage cells which corresponded to the location of the glycogen after fixation. He also stressed the great regularity of orientation of glycogen granules in the kidney tubules of the cat. Similar orientation in the uterine epithelium is described by Bartelmez and Bensley ('32), but these authors

³ This submicroscopic particle, as will be shown later, consists of an aggregate of smaller glycogen units. Upon dispersion, this particle assumes the properties of glycogen, prepared by the method of Pflüger.

state they are uncertain as to whether the granules are preformed or are the result of plasmolysis. However, Lewis and Nordman observed that in the process of staining by iodine vapor the cells in the tissue culture, which previously showed a diffuse browning, would, at times, suddenly develop blebs or granules of brown staining material. Nordman, at times, observed these droplets in the plasma outside the cells. These transformations were only observed during the stage of cell death. This observation might be taken, at first glance, to suggest that all vacuoles are secondary to cell death; however, since iodine-potassium iodide solution is a strong protein coagulator,⁴ these changes may well be the result of iodine coagulation. They do not necessarily account for the vacuolar appearance seen in certain fresh or dried cells.

It is of interest to note that Cori, Cori and Schmidt ('39), found that the glycogen, synthesized by an enzyme prepared from muscle, increased the turbidity of the solution and resulted in a granular precipitate visible under the microscope.⁵

It is clear, therefore, that glycogen exists as a uniformly distributed substance in the liver cell. We have separated it in the form of a submicroscopic particle. Whether it also exists in a microscopically visible form in liver and other cells awaits further study.

SEPARATION OF THE PARTICULATE GLYCOGEN

Adult guinea pigs, allowed excess food for the previous 24-48 hours were killed by a blow on the head. The thoracic cavities were opened, and by way of the descending aorta, the animals were perfused with cold saline. After perfusion, the

⁴ If one adds a solution of I-KI to a liver extract freed of all the microscopic and submicroscopic granules the protein is promptly coagulated and forms a precipitate.

⁵ No granules, however, resulted from glycogen synthesis when the enzyme was prepared from other sources (liver and brain). Since myosin has been shown by Przylecki and Majmin ('34) to form precipitated complexes with glycogen, whereas albumens and globulins, at physiological pH's do not, Przylecki, Andrzejewski and Mystkowski ('35), it is not unlikely that the granular precipitate of glycogen, synthesized by the muscle enzyme is due to contained myosin in the enzyme preparation.

livers were removed and fragmented by forcing them through surgical gauze and finally through bolting silk. This procedure fragments a large percentage of the cytoplasmic membranes of the cells, thus dispersing most of the cytoplasmic constituents. The nuclear membranes, however, are not easily disrupted and most of the nuclei remain intact. A few intact cells also remain. This liver emulsion was suspended in three times its volume of 0.85% sodium chloride and fractionated by differential centrifugation in a cold room at 2–3°C.

The suspension was clarified for two 10-minute intervals at 3000 R.P.M. in an 8-inch angle centrifuge and then for 15 minutes at 6000 R.P.M. The above precipitates⁶ were discarded. The supernatant was then transferred to clean 10 cc. lusteroid test tubes, and centrifuged at 12,000 R.P.M.⁷ for 30 minutes. The precipitate consisted of two parts: (a) a densely packed cake which remains inclined in the tube corresponding to its position on centrifugation—this is the particulate glycogen; (b) a loosely packed, red colored, precipitate which is easily separated from (a) by inverting the test tube. This latter precipitate is a mixture of particulate glycogen and the lipo-protein submicroscopic cell component. The surface of the tightly packed cake is washed twice with saline and then resuspended in saline, allowing 30 minutes for dispersion. It is then recentrifuged at 12,000 R.P.M. for 30 minutes and all but the tightly packed cake is discarded. The above procedure is repeated four times with saline, or with distilled water if desired. This cake upon being dried in a vacuum desiccator yields a pure white powder. Chemical analysis shows this powder to consist mainly of glycogen.

METHODS OF CHEMICAL ANALYSIS

The jelly-like cake was dried in a vacuum desiccator over P_2O_5 for 24–48 hours and then extracted in a Soxhlet extractor for a period of 6 days (48 hours with each of the following:

⁶ These precipitates contained nuclei, mitochondria, and some submicroscopic components.

⁷ This corresponds to a gravitational force of about 12,000 G.

redistilled alcohol, ether and chloroform). The above three extracts were combined, evaporated to dryness and re-extracted with chloroform. The fats go into solution, leaving a considerable residue behind which is water soluble. This residue is composed largely of salts but contains other organic extractives. This method of extraction is fairly efficient as only 0.3% ash remains in the original alcohol-ether-chloroform (A-E-C) insoluble residue.

Water. In some cases the approximate quantity of water in the original jelly was estimated by comparing the chloride concentration of the dried jelly with that of the saline used for extraction. The chlorides were determined gravimetrically. This, of course, assumes that the salts are uniformly distributed throughout all the water of the particle. In other cases the water was determined directly by weighing the jelly before and after drying. Obviously, these methods of water analysis are only approximate. The water percentage does not represent the true water content of the particle; for if we assume that the glycogen particles are spheres and that they are packed as closely as their configuration permits, some of this water lies outside the particle. A calculation shows that 26% of the water would be extra-particulate if the above assumptions were correct.

Fats. The chloroform soluble fraction was dried in a vacuum desiccator for 24 hours and the fats weighed on a micro-balance.

The A-E-C insoluble residue was analyzed for carbon, hydrogen, ash, nitrogen, phosphorus, glucose, and protein. Micro carbon, hydrogen, and ash analyses were performed by Doctor Ma of the Department of Chemistry by the micro Pregl method ('37). The nitrogen was determined by micro Kjeldahl digestion followed by direct Nesslerization as given by Koch ('37). The phosphorus was determined by a modification of the Fiske and Subbarow method ('25), using higher acidity and heat to develop the blue color.

Glycogen. The glycogen was determined as glucose following a 30-minute period of hydrolysis with 3 N. H_2SO_4 . The

glucose was determined by the micro method of Miller-Van Slyke ('36). The glucose value multiplied by the factor 162/180 gives the glycogen content.

Protein. An attempt to determine how much of the total nitrogen represents protein was not entirely successful. Proteins are precipitated by trichloroacetic acid (T. A. A.). If the nitrogen is determined in the T. A. A. filtrate and precipitate, the ratio of protein nitrogen to total nitrogen can be calculated. However, when the percent of protein is very low and is present in a complex, as is the case in our material, not all of the protein is coagulated by cold T. A. A., and part remains in solution. But, if the T. A. A. is heated for 30 minutes in a water bath the protein is aggregated into large clumps. Heating with 4% T. A. A. for 30 minutes hydrolyzes some of the protein.⁸ In spite of this fact, more protein was recovered from the glycogen sample after heating than was recovered from precipitation in the cold. Although the method for protein estimation is inadequate, we can show that the nitrogen in the sample, in large part at least, represents protein nitrogen.

PROPERTIES OF PARTICULATE GLYCOGEN

General physical properties. The moist centrifuged cake has a jelly-like consistency. It is transparent and has a white or faint yellow color. When viewed by reflected light it is moderately opaque. If the cake is allowed to stand for a short time in 0.85% sodium chloride, or water, it disperses and produces a markedly opalescent solution. This solution is fairly stable for many days, but can be precipitated by 50% alcohol. It redisperses upon adding water.

Microscopic properties. A suspension of particulate glycogen, when studied under the microscope with an oil immersion lens shows no structure whatever. When studied with an ultramicroscope, small particles, undergoing rapid Brownian movement, are distinctly visible.

Centrifugability. The particulate glycogen may be completely removed from solution by centrifuging in an angle

⁸ Using similar amounts of crystalline egg albumen, about 25% of the protein is hydrolyzed and appears in the T. A. A. filtrate.

centrifuge for 30 minutes at 12,000 R.P.M. (This corresponds to a centrifugal force of 12,000 G.) When centrifuging commercial glycogen (Pfanstiehl) for the same length of time 99.7% of the glycogen remains in solution. However, the particulate glycogen begins to sediment at speeds as low as 3000 R.P.M. After a 40-minute centrifugation at this speed the glycogen is seen to have settled in a fairly sharp boundary. The upper 10 mm. of the tube showed no opalescence and contained no glycogen, whereas the rest of the tube appeared markedly opalescent. Using the Svedberg ('40), definition of sedimentation constant as the rate of sedimentation expressed as centimeters per second when the sedimenting force is one dyne, we obtain an approximate value of $4,200 \times 10^{-13}$ for particulate glycogen. A comparison with table 1 shows

TABLE 1
Particle weight and sedimentation constants of various proteins.

	$K_s \times 10^{-13}$	M.W. (MILLION)
Serum albumen (horse) ¹	4.5	0.067
Edestin ¹	12.8	0.303
Erythrocrucorin (Planoribis blood) ¹	33.7	1.6
Hemocyanin (Helix Blood) ¹	98.9	6.6
Tobacco Mosaic Virus ²	191-244	15-20
Particulate glycogen	4,200 (approximate)	?

¹ Taken from Schmidt ('38) pp. 393-394.

² Taken from Eriksson-Quensel and Svedberg ('36).

that the particle weight of particulate glycogen is much greater than even that of Tobacco Mosaic virus. It is also of interest to compare the particle weight of the former with glycogen prepared in the usual way. Oakley and Young ('36), estimated by means of an osmotic pressure method, that glycogen prepared by KOH extraction had an average molecular weight of two million. Mystkowski ('37) found that similar preparations of glycogen began to sediment at 17,000 R.P.M. Since the particulate glycogen begins to sediment at 3000 R.P.M., whereas, the same glycogen, after dispersion with KOH, cannot be removed by the maximum speed of our

centrifuge (12,000 R.P.M.), it is clear that the particle represents an aggregate of many smaller glycogen units.

Dispersion of the particulate glycogen. The particulate glycogen can be dispersed by any of the agents commonly used for the separation of glycogen from the cell. Four per cent Trichloroacetic acid or 25% NaOH produced a marked decrease in the turbidity of the solution as well as a loss of the centrifugability. A control test was performed by adding sodium chloride to the particulate glycogen suspension to produce a corresponding increase in density but this showed no loss of centrifugability. Similarly, heating for 2 hours on a steam bath produced a marked decrease in the opalescence of the solution and 94% of the glycogen particles were dispersed. The remaining 6% could still be recovered by the centrifuge. Incubation for 2 hours at 37°C. showed no dispersion of the particle.

CHEMICAL PROPERTIES

The glycogen gives a positive Molish test, and a brown to red color with iodine. The latter disappears upon heating and reappears upon cooling. It reduces Fehlings solution only after hydrolysis.

TABLE 2
Analysis of the moist product.

	SAMPLE ¹ C	SAMPLE ² D
	%	%
Water (approximate value)	73	73
Solids	27	27

¹ H₂O estimated from salts.

² H₂O estimated by drying.

TABLE 3
Analysis of the dried material.

	SAMPLE A	SAMPLE C
	%	%
Salts and other water soluble extractives	1.50 ³	2.57
Fat (chloroform soluble)	0.16	0.165
Residue (A-E-C insoluble)	98.34	97.26

³ The particulate glycogen in this case was washed once in distilled H₂O before drying.

It is to be noted that sample A (see table 4) which was dried in a vacuum desiccator, after extraction of the fats and salts, still showed evidence of residual moisture content, for the percentage of hydrogen was high. However, upon heating sample C at 100°C. for 24 hours and then drying in a vacuum desiccator a considerable portion of the water was lost, as evidenced

TABLE 4
Analysis of the alcohol-ether-chloroform insoluble residue.

	SAMPLE A (DRIED OVER P_2O_5 IN VACUUM DESICCATOR)	SAMPLE C (DRIED AT 100°C. 2 HRS. AND THEN PLACED IN VACUUM DESICCATOR)	$C_6H_{10}O_5$	COMMERCIAL GLYCOGEN DRIED IN VACUUM DESICCATOR
	%	%	%	%
Carbon	41.67	41.50	44.56	...
Hydrogen	7.78	6.46	6.22	...
Ash		0.37		...
Glycogen	92.2	93.5		93.2
Phosphorus	0.033	0.019		...
Nitrogen	0.23	0.17		...
Protein nitrogen (T.A.A. ppt.)		0.102		...
Protein (maximum)		1.07		...
Protein (minimum) ¹		0.86		...

¹ Determined by correcting T.A.A. ppt. nitrogen for hydrolysis and multiplying by 100/15.9.

by much lower hydrogen values. Slater ('24) actually succeeded in preparing a mono-hydrate of glycogen ($C_6H_{10}O_5 \cdot H_2O$)_n which when dried in a vacuum desiccator over $CaCl_2$ gave up only 50% of its moisture content. The resulting hemi-hydrate loses about 30% more of the original water content upon heating at 110°C. for 1-2 hours. It takes 9 days, however, to lose most of the remaining water.

From some of our experimental data it seems that this peculiar hydration property may be related, in part, to the absence of salts. The original particulate glycogen was separated from salt solution and was dried in a vacuum desiccator

over P_2O_5 . However, after extraction with alcohol, ether, and chloroform, it showed a 6.95% increase in weight when dried under identical conditions. Upon heating at $100^\circ C$. for 24 hours part of this apparent gain in weight was lost. The extraction removes most of the salts. The evidence previously presented, with respect to the hydrogen content, suggests that this increase represents water. By electrophoresis studies glycogen has been shown to have a negative charge (Samec and Isajevic, '23; and McBride, '29). In the presence of salts, the ions may serve to maintain electrostatic equilibrium. In the absence of salts, however, utilization of the water dipoles may be required to accomplish this purpose and hence the difficulty in removing the residual water.

THE PREEXISTENCE OF THE SUBMICROSCOPIC PARTICULATES IN THE CELL

Do these isolated glycogen particulates exist as such in the cell or are they the artificial result of the procedure used in separating them? If these particles were artificially formed by the conditions of the separation, addition of dispersed glycogen to the supernatant (out of which the glycogen particulates were originally obtained) should result in aggregation and development of more particles. Both particulate glycogen, dispersed by heat, and commercial glycogen were added to the supernatant and incubated for 3 hours at $37^\circ C$. and $0^\circ C$. There was no evidence of particulate formation — that is no change in turbidity of the solution and no appearance of particulates upon centrifugation.

The conditions of the separation involve no drastic alteration of pH or protein precipitating agents. The only change is from the ionic environment of the cell to that of 0.85% NaCl. An attempt was made to separate the particulate glycogen in salt solutions approaching intracellular fluid.⁹ The following solutions were used: (a) KCl-MgCl₂-phosphate

⁹ Hastings and his associates have suggested that intracellular fluid is rich in potassium, magnesium, and phosphate and poor in chloride. See Manery and Hastings ('39).

mixture, and (b) potassium phosphate buffer, pH 7.2. Particulate glycogen was separated in both cases when these salt solutions were used for fragmentation of the liver cells.

The above findings, taken with the fact that particulate glycogen is dispersed by all the agents commonly used to separate glycogen from the cell (heat, trichloroacetic acid, and KOH), strongly suggests that it is present as such in the cytoplasm of the cell. The demonstration of the centrifugability of glycogen within the liver cell may furnish more direct proof of the preexistence of the glycogen particles. This experiment will be undertaken.

DISCUSSION

Water content. The separation of the particulate glycogen containing water is of interest because of the various reports in the literature on this question. Fenn ('39) reviewed the literature on this subject. He presents good evidence that glycogen is stored in the cell along with water, potassium, and acid soluble phosphate. This is concluded from the fact that as the glycogen content of the whole liver increases, the water content, potassium, and acid soluble phosphate show a parallel increase, whereas the protein content remains unchanged. The actual percentages, however, of water, potassium, and acid soluble phosphate remain relatively unchanged, whereas the percentage of protein decreases. Glycogen storage is, of course, associated with an increase in weight of the liver. From this data Fenn concluded that for each gram of glycogen, 2-3 gm. of water were also stored in the liver.

Protein content. The isolation of a glycogen particulate complex containing a small amount of protein is of special interest. Combinations of protein and glycogen have been known for some time. Przylecki and Majmin ('34), and Mystkowski, Stiller and Zysman ('35), showed combinations of glycogen with myosin and myoglobin. Mystkowski ('37), by means of an ultracentrifugal study, has shown a combination of serum globulin and glycogen; the addition of glycogen produces a 20% apparent increase in the molecular weight of

the globulin. He failed to show any combination with albumen. Willstätter and Rohdewald ('34), who reviewed the early literature on this subject, found, by isoelectric precipitation of a liver extract, that they could obtain a protein fraction containing glycogen. Aubel, Reich, and Lang ('38), were able to isolate a similar complex by ammonium sulphate precipitation of the proteins. This contained 20% glycogen. Wajzer ('39), in studying the amount of glycogen protein complex in the liver of frogs, was able to recover from 46–94% of the total liver glycogen by trichloroacetic acid precipitation of liver extracts. None of these authors, however, report a glycogen percentage in any way approaching the value of our complex.

If all the nitrogen in our preparation represents protein, the total protein amounts to only 1–1.4%. It would appear, at first glance, that this might be a contaminant — especially since this particulate glycogen must be separated from a second submicroscopic lipo-protein complex. But the percentage of fat in the glycogen sample amounts to only 0.16%. The lipo-protein complex contains about 40–50% fat and 50–60% protein. Therefore, if all the fat present were due to the lipo-protein complex contamination, the protein contamination would be approximately 0.16%. This figure is but a small fraction of the actual protein found.

This particulate glycogen is dispersed by heat, trichloroacetic acid, and alkali. None of these agents is said to affect glycogen, whereas they all radically modify proteins. This suggests, therefore, that the protein content, although small, plays an important role in the particulate structure. It is not unlikely that the protein complexes isolated by the above authors are mixtures containing the particulate glycogen and protein.

Significance of particulate glycogen in the problem of insoluble glycogen. The protein content assumes further significance when considering the problem of the solubility of glycogen in various tissues. Ehrlich, as early as 1883, was aware that the glycogen of different tissues dissolves at different

rates. For example, he states that the glycogen of liver sections readily dissolves in water; the glycogen in cartilage is dissolved with much greater difficulty; that in stratified epithelium is not dissolved at all. Not only did he point out that a second substance, which he called "Träger Substance" might be responsible for this variation in solubility, but he suggested that this might be a protein. Külz (1886) made a similar suggestion. The difficulty of removing all the glycogen from muscle by aqueous extraction is a well known fact.

Fränkel (1892), stated that freshly ground liver, or liver which was coagulated with alcohol, dried and powdered, gave no glycogen when extracted with cold water. If the same preparations were extracted with hot water, acids, or salts of heavy metals, a cloudy solution containing glycogen was obtained. He considered this data as proof of a protein complex and of the liberation of glycogen by protein coagulating agents. Saake (1892), however, pointed out that these results could also be interpreted on the basis of alterations of permeability of the cell wall brought about by the above agents.

The separation of particulate glycogen throws more light on the problem of insoluble glycogen. Whether or not these particles can get out of the cell would depend upon the permeability of the cell wall. By our method of fragmenting liver, most of the cytoplasmic membranes are broken. Hence, the particulates are liberated, even in ice water. However, if the liver is simply ground large numbers of cells would remain intact and cold water could not be expected to remove the glycogen. The application of heat and acid not only alters the permeability, but also dissociates the particle. This liberates the glycogen from the cell. Other cells such as cartilage and stratified epithelium may hardly be broken at all by grinding, and consequently give little glycogen by simple aqueous extraction.

The case of muscle is especially interesting. It is relatively difficult to break up muscle fibers mechanically. If the particulates are present in muscle as well as in liver they might not easily be liberated. Furthermore, since the myosin

extracted from the cell is precipitated at pH 6.9–7, it is easy to understand why, at the pH of the cell, the cytoplasm of broken muscle cells shows little tendency to disperse. Meyerhoff ('30) pointed out that in frog muscle only one-sixth of the muscle glycogen could be obtained in the pressed muscle juice. Furthermore, Przylecki and Majmin ('34), and Mystkowski, Stiller and Zysman ('35), pointed out that myosin forms definite complexes with glycogen and that preparations of myosin and myoglobin-X contain measurable quantities of glycogen. These authors state that in the muscle cell the myosin and myoglobin are intimately associated with the glycogen. It remains to be investigated whether or not submicroscopic aggregates of glycogen exist in muscle and what their relationship is to myosin and the structure of muscle.

Phosphorus content. Part of the phosphorus content of our preparation may also be explained on the basis of the protein content. Other proteins which were separated from guinea pig liver contain phosphorus. Sahyun and Alsberg ('30) reported glycogen samples giving no phosphorus directly, but giving about 0.03% after hydrolysis. McDowell ('22) was unable to free glycogen from phosphorus by prolonged electrodialysis. Hassid and Chaikoff ('38), using purified glycogen preparations, obtained by trichloroacetic acid precipitation, report a much lower percentage of phosphorus (0.001–0.01%). From our previously mentioned experience in attempting to determine the protein nitrogen by trichloroacetic acid precipitation it is clear that all the protein cannot be removed from the glycogen by cold trichloroacetic acid.

Significance of particulate glycogen in glycogen storage and the regulation of blood sugar. It is clear that glycogen exists in the liver in the form of submicroscopic particles. These particles, which are aggregates of smaller glycogen units, are very stable at 37°C. but they can be dispersed by drastic treatment, such as prolonged heating at 100°C., strong alkali or acid. Although they contain a very small amount of protein, this protein may be of great importance in the maintenance of glycogen in the particulate state inasmuch as all the agents

which disperse the particulate glycogen markedly alter protein. None of these is thought to alter the properties of glycogen.

It is clear that, if this protein, or some other agent, combines with the dispersed glycogen as the latter is synthesized in the liver cell, the glycogen will be removed from solution by the formation of particulates. By the law of Mass Action the enzymatic reaction



would be shifted in favor of glycogen synthesis, therefore, facilitating glycogen storage in the liver. The concentration of glucose in the liver cell would be diminished. This would favor the removal of glucose from the blood stream and a consequent lowering of blood sugar.

If this coacervating agent were insulin, or, if insulin catalyses this reaction, it could explain one mechanism of insulin action, for we know that insulin effectively lowers the blood sugar and facilitates glycogen storage. Whether it is insulin or not, a simple defect in the mechanism of particulate glycogen formation within the liver cell would result in a decrease in glycogen storage and an elevation of the blood sugar. If the renal threshold were exceeded a glycosuria would develop and a "diabetic like" state would result. All of this could be the result of a primary defect within the liver cell — i.e., a liver diabetes. Thus one type of diabetes could be the result of a defect in the regulatory mechanism within the liver cell and still could be associated with a "normal" utilization of sugar, though at a higher blood level, and "normal" rate of conversion of fats and proteins into sugar. If insulin were the agent which controls particulate glycogen formation, then pancreatic diabetes could be the result of a primary failure in this regulatory mechanism. This is in direct contrast to the non-utilization and overproduction theories for the cause of pancreatic diabetes.

Investigations are now in progress to determine the nature of the protein contained in particulate glycogen, the factors

¹⁰ The enzyme glycogen phosphorylase, which catalyzes this reaction has been isolated by Cori, Cori and Schmidt ('39).

involved in particulate glycogen formation, and the state of glycogen in various types of experimental animals.

CONCLUSIONS

1. A submicroscopic complex of glycogen has been isolated from the liver of guinea pigs by means of a high speed centrifuge.

2. This complex contains a high percentage of water and lends support to the concept of storage of water with glycogen in the cell.

3. It contains 92–93.5% of glycogen on a dry weight basis.

4. It contains a small percentage of protein (approximately 1%) but this may play a very important role in the maintenance of the complex.

5. It contains a small amount of nitrogen and phosphorus. Most, if not all, of the nitrogen as well as part of the phosphorus is due to the protein content.

6. The particles are stable at 37°C. but may be dissociated into smaller units by heat, trichloroacetic acid and KOH (common agents used in glycogen preparation).

7. The significance of the particulate glycogen in the process of glycogen storage in the liver and the regulation of blood sugar has been discussed.

I should like to express my deep appreciation to Prof. R. R. Bensley for his suggestions, criticisms, and constant encouragement throughout this work.

LITERATURE CITED

- AFANASSJEW, M. 1883 Über anatomische Veränderungen der Leber während verschiedener Thatigkeitszustände. *Archiv. f. d. ges. Physiol.*, vol. 30, p. 385.
- AUBEL, E., W. S. REICH AND F. M. LANG 1938 Sur l'état du glycogène dans le foie. *Compt. rend. Acad. d. Sc.*, vol. 206, p. 777.
- BARFURTH, DIETRICH 1885 Vergleichend-histochemische Untersuchungen über das Glycogen. *Archiv. f. mikros. Anat.*, vol. 25, p. 269.
- BARTELMEZ, G. W., AND C. M. BENSLEY 1932 In *Special Cytology*, edited by E. V. Cowdry, vol. III, p. 1525. Paul B. Hoeber, Inc., New York.

- BENSLEY, R. R. 1937 On fat distribution in mitochondria of guinea pig liver. *Anat. Rec.*, vol. 69, p. 341.
- BENSLEY, R. R., AND N. L. HOERR 1934 Studies on cell structure by freezing-drying method; preparation and properties of mitochondria. *Anat. Rec.*, vol. 60, p. 449.
- BERNARD, CLAUDE 1857 Sur le mécanisme physiologique de la formation du sucre dans le foie. *Compt. rend. Acad. d. Sc.*, vol. 44, p. 578.
- 1859 Mémoire sur une nouvelle fonction du Placenta. *Brown-Séquard, J. de Physiol.*, vol. 2, p. 31.
- BEST 1903 Über Glykogen, insbesondere seine Bedeutung bei Entzündung und Eiterung. *Beitr. z. path. Anat. u. z. allg. Path.*, vol. 33, p. 585.
- BOCK, C., AND F. A. HOFFMANN 1872 Ueber das microchemische Verhalten der Leberzellen. *Virchows Arch. f. path. Anat.*, vol. 56, p. 201.
- CLAUDE, A. 1940 Particulate components of normal and tumor cells. *Science*, vol. 91, p. 77.
- CORI, G. T., C. F. CORI AND G. SCHMIDT 1939 Role of glucose-1-phosphate in formation of blood sugar and synthesis of glycogen in liver. *J. Biol. Chem.*, vol. 129, p. 629.
- EHRlich, P. 1883 Appears as an appendix in the article by F. T. Frerichs. Über den plötzlichen Tod und über das Coma bei Didabetes (diabetische Intoxication). *Ztschr. f. klin. Med.*, vol. 6, p. 35.
- ERIKSSON-QUENSEL, I. B., AND T. SVEDBERG 1936 Sedimentation and electrophoresis of the Tobacco-Mosaic virus protein. *J. Am. Chem. Soc.*, vol. 58, p. 1863.
- FENN, W. O. 1939 The deposition of potassium and phosphate with glycogen in rat livers. *J. Biol. Chem.*, vol. 128, p. 297.
- FISCHER, G. 1904 Über die Verteilung des Glykogens in verschiedenen Arten experimenteller Glykosurie. *Beitr. z. path. Anat. u. z. allg. Path.*, vol. 36, p. 273.
- FISKE, C. H., AND Y. SUBBAROW 1925 The colorimetric determination of phosphorus. *J. Biol. Chem.*, vol. 66, p. 375.
- FRÄNKEL, S. 1892 Studien über Glykogen. *Archiv. f. d. ges. Physiol.*, vol. 52, p. 125.
- GERSH, I. 1938 Results quoted in Maximow, A. A., and W. Bloom. *Textbook of Histology*. W. B. Saunders Company, Philadelphia, 1938.
- GIERKE, E. 1905 Das Glykogen in der Morphologie des Zellstoffwechsels. *Beitr. z. path. Anat. u. z. allg. Path.*, vol. 37, p. 502.
- HASSID, W. Z., AND I. L. CHAIKOFF 1938 The molecular structure of the liver glycogen of the dog. *J. Biol. Chem.*, vol. 123, p. 755.
- HEIDENHAIN, R. 1883 In L. Hermann's *Handbuch der Physiologie*, vol. 5. Leipzig, F. C. W. Vogel, 1883.
- KABAT, E. A. 1941 Association of phosphatase with material in kidney sedimentable at high speed and its liberation by autolysis. *Science*, vol. 93, p. 44.
- KOCH, F. C. 1937 *Practical Methods in Biochemistry*, William Wood and Co., Baltimore, 1937.
- KÜLZ, E. 1881 Kommt Glycogen in der ersten Anlage des Hühnchens vor? *Arch. f. d. ges. Physiol.*, vol. 24, p. 61.

- KÜLZ, R. 1886 Zur quantitativen Bestimmung des Glykogens. *Ztschr. f. Biol.*, vol. 22, p. 161.
- LAZAROW, A., AND E. S. G. BARRÓN 1941 The oxygen uptake of mitochondria and other cell fragments. *Anat. Rec.*, vol. 79, no. 3, Supp. no. 2, p. 41.
- LEWIS, M. R. 1921 The presence of glycogen in the cells of embryos of *Fundulus heteroclitus* studied in tissue culture. *Biol. Bull.*, vol. 41, p. 241.
- MANERY, F. J., AND B. A. HASTINGS 1939 The distribution of electrolytes in mammalian tissues. *J. Biol. Chem.*, vol. 127, p. 657.
- MARCHAND, F. 1885 Über eine Geschwulst aus quergestreiften Muskelfasern mit ungewöhnlichem Gehalte an Glykogen, nebst Bemerkungen über das Glykogen in einigen fötalen Geweben. *Virchow's Archif. f. path. Anat.*, vol. 100, p. 42.
- MCBRIDE, J. J. 1929 A chemical study of glycogen and its preparation. Columbia University thesis, New York City.
- MCDOWELL, M. 1927 Glycogen of edible mussel, *Mytilus edulis*. *Proc. Soc. Exper. Biol. and Med.*, vol. 25, p. 85.
- MEYERHOFF, O. 1930 Die chemischen Vorgänge im Muskel und ihre Zusammenhang mit Arbeitsleistung und Wärmebildung. *J. Springer, Berlin.*
- MILLER, B., AND D. D. VAN SLYKE 1936 A direct microtitration method for blood sugar. *J. Biol. Chem.*, vol. 114, p. 583.
- MYSTKOWSKI, E. M. 1937 Ultracentrifugal studies of compounds of proteins with polysaccharides. Compounds between proteins and glycogen. *Biochem. J.*, vol. 31, p. 716.
- MYSTKOWSKI, E. M., A. STILLER AND A. ZYSMAN 1935 Über den Zustand des Glykogens in Muskel. *Biochem. Ztschr.*, vol. 281, p. 231.
- NORDMAN, M. 1929 Wachstum und Stoffwechsel der Leberzellen in der Gewebeskulture. *Arch. f. exper. Zellforsch.*, vol. 8, p. 371.
- OAKLEY, H. B., AND F. G. YOUNG 1936 The osmotic pressure of glycogen solutions. *Biochem. J.*, vol. 30, p. 868.
- PREGL 1937 Roth-Daw Quantitative Organic Microanalysis of F. Pregl. Blakiston's Son and Co., Philadelphia.
- PRZYLECKI, S. J., H. ANDRZEJEWSKI AND E. MYSTKOWSKI 1935 Über Sole von Polysacharoproteiden. *Kolloid Ztschr.*, vol. 71, p. 325.
- PRZYLECKI, S. J., AND R. MAJMIN 1934 Über Polysacharoproteide. II Teil, Verbindungen mit denaturierten Proteinen. *Biochem. Ztschr.*, vol. 271, p. 168.
- 1934 Über die Verbindung Myosin-Polysaccharid. *Biochem. Ztschr.*, vol. 273, p. 262.
- ROUGET, C. 1859 De la substance amylacée amorphe dans les tissue des embryons des Vertébrés. *Compt. rend. Acad. d. Sc.*, vol. 48, p. 1018.
- SAAKE, W. 1892 Studien über Glykogen. *Ztschr. f. Biol.*, vol. 29, p. 429.
- SAHYUN, M., AND G. L. ALSBERG 1930 On rabbit liver glycogen and its preparation. *J. Biol. Chem.*, vol. 89, p. 33.
- SAMEC, M. M., AND V. ISAJEVIC 1923 Sur la composition du glycogène. *Compt. rend. Acad. de Sc.*, vol. 176, p. 1419.
- SCHIFF, M. 1857 Mitteilung. *Arch. f. physiol. Heilkund.*, vol. 1, p. 263.

- SCHMIDT, C. L. A. 1938 The Chemistry of the Amino Acids and Proteins. Charles C. Thomas, Springfield, Illinois.
- SLATER, W. K. 1924 A redetermination of the heat of combustion of glycogen with special reference to its physiological importance. *Biochem. J.*, vol. 18, p. 621.
- SVEDBERG, T. 1940 Svedberg, T., and K. O. Pedersen, *The Ultracentrifuge*. Clarendon Press, Oxford.
- WAJZER, J. 1939 Formation d'un glycogène-protéide au cours de la synthèse du glycogène. *Compt. rend. soc. de Biol.*, vol. 130, p. 1119.
- WILLSTÄTTER, R., AND M. ROHDEWALD 1934 Über den Zustand des Glykogens in der Leber, im Muskel und Leukocyten. *Ztschr. f. physiol. Chem.*, vol. 225, p. 103.

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